STEPHEN S. TOBE* and KENNETH G. DAVEY

AUTORADIOGRAPHIC STUDY OF PROTEIN SYNTHESIS IN ABDOMINAL TISSUES OF GLOSSINA AUSTENI

ABSTRACT. Protein synthesis at various times during the pregnancy cycle of G. austeni was determined by autoradiographic measurement of the incorporation of H\textsuperscript{3}-leucine and H\textsuperscript{3}-tyrosine into the cells of the fat body, oenocytes, milk gland and epidermis. The rate of utilization of these molecules is such that the labelled pool in the haemolymph is depleted before 0.5 hr after injection. The incorporation of both amino acids into fat body and oenocytes is high at eclosion and just after larviposition, with the incorporation of tyrosine by the oenocytes being much higher than that in the fat body. The same pattern of incorporation is observed in the epidermal cells. Label also appears in the endocuticle during the first 10 days of adult life. Except during the first 4 days following emergence, the incorporation of the two amino acids into the milk gland is very high, with periods of less intense protein synthesis at about the time of larviposition. The milk gland represents a highly efficient secretory system, with a t\textsubscript{1/2} of less than 30 min.

Introduction

In a previous paper (Tobe and Davey, 1974a) we have described the synthesis of nucleic acids in various abdominal tissues of Glossina austeni during the first two pregnancy cycles. Briefly, that study revealed cycles of RNA synthesis in milk gland, fat body and oenocytes which were correlated with the cyclical secretory activity of the milk gland. These studies on nucleic acid synthesis were a logical preliminary to the present work, which represents an analysis of protein synthesis in the female of G. austeni.

While protein synthesis in Glossina has not been studied previously, some aspects of amino acid metabolism have received attention. Thus, it is clear from the work of Bursell (1960, 1963, 1967) that proline is used as an energy source, while this is not the case in non-haematophagous diptera (Bursell, 1960; Chen, 1966). Tyrosine appears to play an important role in tsetse flies. Thus, tyrosine, which is abundant in the pupa and pharate adult of G. palpalis (Balogun, 1969; Balogun et al., 1969), is not present in adult females (Balogun, 1971). Tyrosine from the blood meal disappears from the haemolymph soon after feeding (Bursell, 1963), and is present in large quantities in the gut contents of G. morsitans (Cmelik et al., 1969).

Because of this unusual predominance of tyrosine in the 'milk' of Glossina, the present study has investigated the incorporation of radioactive tyrosine into tissue proteins and secretory proteins by autoradiographic techniques. The incorporation of labelled leucine has also been followed because leucine has been generally used for investigations on protein synthesis. The present paper is one of a series which explores the transfer of nutrients in female Glossina, with particular emphasis on amino acids and proteins.

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Materials and Methods

G. austeni were maintained on rabbit blood after the method described by Jordan et al. (1967, 1968) with the modifications described by Tobe and Davey (1971).

For autoradiography, flies of various ages were injected in the thorax with 1 µl (1 µC) of either L-tyrosine-3, 5-H³ (specific activity 220 mCi/mg, Amersham/Searle) or L-leucine-H³(G) (specific activity, 3·0 mCi/mg, Amersham/Searle), and incubated for 0·5, 2 or 4 hr. Abdomens were fixed and prepared for autoradiography as described previously (Tobe and Davey, 1974a).

For quantification of the results, grain counts were performed on the autoradiographs. Ten random sections of 100 µm² of each tissue for each animal were counted under oil immersion at x 1000 to determine cytoplasmic label. All figures for autoradiographs have been based upon physiological age (see Tobe and Davey, 1972, 1974a, b). In the figures, E = ecdisys, O = ovulation, L₁ and L₂ = first and second larviposition.

Results

Rates of utilization of H³-tyrosine and H³-leucine

In order to appreciate the dynamics of amino acid utilization in Glossina, it is first necessary to estimate the rates of utilization of these compounds. These estimates will also give some indication of the rates of utilization of the injected labelled amino acids used in the present study.

It is known that 4·5 ng of H³-tyrosine were injected into each fly, as determined from the specific activity. The results of Cmelik et al. (1969) indicate that 15·3% of the milk secretion in the larval gut of G. morsitans is protein, peptide and amino acid and of this, 16·9% is tyrosine. Thus, if G. austeni elaborates 15 mg of secretion during each pregnancy cycle (see Tobe and Davey, 1972) and the figures of Cmelik et al. (1969) on G. morsitans can be applied to G. austeni, we find that 15·3/100 x 15 x 16·9/100 = 388 µg of tyrosine are utilized during each pregnancy cycle. Now if the milk gland elaborates secretion over a period of 7 days (see Tobe et al., 1973), therefore 388/7 = 55·5 µg of tyrosine are utilized each day or 55·5/24 = 2·3 µg/hr. It is therefore apparent that the pool of 4·5 ng of labelled tyrosine would be utilized in a matter of minutes during the period of elaboration of secretion, assuming that the rate of tyrosine utilization is constant during the 7-day period. In essence, then, the system is rapidly self-chasing during this period, due to the high rate of tyrosine utilization.

From the data on the apparent haemolymph volume of G. austeni after injection with C¹⁴-inulin (Tobe and Davey, 1972), it is known that the inulin is uniformly distributed through the haemolymph within an hour after injection. Therefore, although the milk gland is capable of utilizing all of the injected tyrosine within a matter of minutes, utilization may in fact occur over a longer period, perhaps as much as 1 hr, due to the possibility that the label may not have been uniformly dispersed in the haemolymph. However, the utilization of the H³-tyrosine can occur before complete dispersion and, since the autoradiographs of flies sacrificed 0·5 hr after injection show uniform distribution throughout the abdomen, it can be safely assumed that utilization of the H³-tyrosine occurs in less than 0·5 hr.

Similar calculations, using the results of Cmelik et al. (1969) can be performed on the leucine pool. Thus, leucine and isoleucine represents 8·5% of the protein, peptide and amino acid in the milk of G. morsitans and therefore 15·3/100 x 15 x 8·5/100 = 184 µg of leucine are utilized in the 7-day period of milk secretion. This represents a rate of leucine utilization of 1·1 µg/hr. Since it is known that 330 ng of H³-leucine were injected, all of the labelled leucine would be utilized within 0·5 hr after injection, assuming that the rate of utilization is constant. This also assumes that there is no synthesis of leucine from other precursors in the milk gland.

These estimates on the rate of utilization of amino acids assume that the rate is constant throughout the pregnancy cycle. Since it is known that the rate of secretion of the milk is cyclical this assumption is perhaps questionable. However, it is clear that the rate of utilization of tyrosine and leucine, while at times slower than indicated, is still very rapid indeed.

It should be noted that the labelled molecules are immediately ‘diluted’ by unlabelled
molecules upon injection. As uptake of both labelled and unlabelled molecules continues, the proportion of labelled molecules remaining in the haemolymph will decrease. Thus the fact that uptake of amino acids is a continuous rather than a quantized process will serve to increase the effective pulse duration, suggesting that the pulse time may be somewhat longer than that postulated above. However, the fact that the injected quantity of $\text{H}^3$-tyrosine is only 0.2% of that utilized in 1 hr indicates that even though there may be a slow 'decay' of labelled molecules from the haemolymph, total removal of labelled molecules probably occurs well before 0.5 hr after injection.

\textit{Incorporation of $\text{H}^3$-tyrosine and $\text{H}^3$-leucine by fat body and oenocytes}

Incorporation of $\text{H}^3$-leucine into proteins of fat body and oenocytes after 0.5, 2 and 4 hr incubation is shown in Figs. 1, 2 and 3 respectively. Certain interesting points are immediately apparent from an examination of these figures. Incorporation of leucine into the proteins of both oenocytes and fat body is high on only two occasions during the first two pregnancy cycles—at eclosion and just after the first larviposition. In general, the levels of incorporation are identical for the three periods of incubation. Indeed, the only time at which the levels of incorporation differ with time of incubation is during the period following eclosion. Thus, at emergence the levels of incorporation of $\text{H}^3$-leucine increase slightly as the incubation
time increases and at 4 days post-emergence (6 days before ovulation), incorporation after 4 hr of incubation is considerably higher than at other incubation times.

Four possible factors may interact to explain the fact that the levels of incorporation of $^3$H-leucine into tissue protein are almost identical for the three incubation periods. (i) The pool of labelled leucine may be depleted by 30 min after injection. (ii) The label may represent in some part incorporation into cellular machinery, for example, ribosomal proteins, enzymes and nucleoproteins. If this is so, then the life span of these macromolecules must be greater than 4 hr since there is no change in incorporation level over the 4 hr incubation period. (iii) Many proteins may be and probably are released into the haemolymph as soon as they are synthesized. However, those proteins which are involved in cellular processes and those destined for storage and later release are retained in the cytoplasm. The fraction of protein destined for storage appears to be quite low. (iv) If the cytoplasmic free leucine pool is large, the gradual 'dilution' of this pool by labelled molecules would alter only slightly the apparent incorporation level. This will be particularly true if the haemolymph leucine pool is also large.

There is some possibility that various combinations of the above explanations are operative at any given time. Possibilities (i)–(iii) will be operative at times of maximum incorporation while (ii)–(iv) may be operative at other times. Thus, at times of maximum incorporation, the size of the labelled pool

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**Fig. 4.** Incorporation of $^3$H-tyrosine into fat body (triangles) and oenocytes (open circles) during the first two pregnancy cycles of female *G. austeni* fixed 0.5 hr after injection. Each point represents the mean of counts on one or two animals. Range of individual counts was less than ± 7%.

**Fig. 5.** As for Fig. 4, but for flies fixed 2 hr after injection.

**Fig. 6.** As for Fig. 4, but for flies fixed 4 hr after injection.
and its subsequent depletion may be the limiting factor, while at other times, the size of the unlabelled pool may be the limiting factor.

The incorporation of $\text{H}^3$-tyrosine into the proteins of the fat body and oenocytes, 0.5, 2 and 4 hr after injection, is shown in Figs. 4, 5 and 6 respectively. The pattern of incorporation is similar to that observed for $\text{H}^3$-leucine, with levels of incorporation by the fat body and oenocytes closely paralleling one another. There are, however, some marked differences. Incorporation by the oenocytes, particularly around the time of the first larviposition, is much higher than that observed in the fat body. Incorporation at the time of the second larviposition increases dramatically in both fat body and oenocytes. The levels of incorporation are similar for the three incubation periods at most times during the first two pregnancy cycles, with the exception of the time of emergence and the time of the first cycle of pregnancy (in the case of oenocytes). Finally, although the specific activity of the $\text{H}^3$-tyrosine is 73 times that of the $\text{H}^3$-leucine, the levels of incorporation are similar.

Figs. 7 and 8 show typical micrographs of autoradiographs of fat body and oenocytes at times of high incorporation (Fig. 7) and low incorporation (Fig. 8) following injection with labelled leucine. The differences are striking.

**Incorporation of $\text{H}^3$-tyrosine and $\text{H}^3$-leucine by epidermal cells**

Incorporation of $\text{H}^3$-leucine and $\text{H}^3$-tyrosine at 0.5, 2, and 4 hr after injection into epidermal cells of the ventral abdominal cuticle of female *G. austeni* during the first two pregnancy cycles is shown in Figs. 9 and 10 respectively. These curves closely parallel one another and the curves for fat body and oenocytes incorporation (see Figs. 1–6). Once again levels of incorporation are the same at each time period for both amino acids, supplying further evidence for the fact that the labelled pool, at most times, is depleted within 0.5 hr after injection. The periods of maximum incorporation occur at emergence and just after the time of the first larviposition, and coincide closely with the periods of intense RNA synthesis reported previously (Tobe and Davey, 1974a).

Epidermal cells of the ventral abdominal cuticle, for the first 4 days of adult life, appear to be accumulating protein. During this period, depletion of the pool may require longer than 0.5 hr, since the levels of incorporation increase over the 4 hr incubation period. This accumulation is in agreement with previous observations.
with the work of Tobe and Davey (1972) who observed that the intensity of staining with protein stains increases over the corresponding period.

In the case of the tyrosine, large amounts of label also begin to appear in the endocuticle of newly emerged flies and label continues to be incorporated into the cuticle until 1–2 days after the first ovulation (see Fig. 11). Although some label is apparent in the endocuticle of flies injected with \( ^3H \)-leucine at eclosion, significant quantities are not incorporated until 8 days after emergence. This supports the histological observations on the deposition of endocuticle during the first 10 days of adult life (Tobe and Davey, 1972).

**Incorporation of \( ^3H \)-tyrosine and \( ^3H \)-leucine by the milk gland**

Incorporation of \( ^3H \)-tyrosine and \( ^3H \)-leucine into protein of the milk gland is shown in Tables 1, 2 and 3. For the purposes of evaluation, the milk gland has been divided into two portions (see Tobe *et al.*, 1973): the proximal tubules, and the distal tubules, and incorporation into each portion has been determined. The distribution of the label in each portion of the milk gland has been expressed as label appearing in the cytoplasm of the secretory cells and label appearing in the secretory vacuoles (see Tobe *et al.*, 1973, for a histological description of the milk gland). Using this method, the movement of label through the cytoplasm into the secretory vacuoles may be conveniently followed.

In general, incorporation of both amino acids by the milk gland is high throughout the observed pregnancy cycles. However, the level of incorporation by both portions of the milk gland during the first 4 days after emergence is quite low (Table 1), reflecting the low levels of growth of the tubules at this time (Tobe *et al.*, 1973). It is of interest that levels of incorporation during the first 8 days of adult life increase between 0·5 and 4 hr (Table 1). This once again suggests that the pool of labelled precursor has not been depleted during the incubation period.

The situation 1 day after the first larviposition seems to differ from that at the beginning of adult life. Table 1 also shows the incorporation of \( ^3H \)-leucine and \( ^3H \)-tyrosine into the two portions of the milk gland, 1 day after the first larviposition. The levels of incorporation for proximal and distal tubules appear to be approximately
equal. The relatively constant incorporation level during the incubation period with H\(^3\)-leucine indicates that the H\(^3\)-leucine pool may not be depleted within 0.5 hr after injection. On the other hand, the situation for H\(^3\)-tyrosine appears to be rather different. The level of incorporation is virtually identical at 0.5 and 2 hr but increases between 2 and 4 hr (see Table 1). This suggests that the H\(^3\)-tyrosine is taken up and stored by some other tissue (perhaps the oenocytes), and subsequently released as either amino acid or peptide between 2 and 4 hr after injection, to be taken in turn by the milk gland. The H\(^3\)-tyrosine must be released as either amino acid or peptide because it has been demonstrated previously (Tobe et al., 1973) that the secretory cells of the milk gland do not take up exogenous protein from the haemolymph.

The movement of labelled protein through the milk gland during the 4 hr of incubation at certain stages of the first two pregnancy cycles is shown in Table 2. In general, label appears to decrease in the cytoplasm and increase in the secretory vacuoles during the incubation periods. This is further evidence for the suggestion that the labelled pool is depleted within 0.5 hr after injection and clearly demonstrates that during the periods of milk elaboration at least, we are dealing with a self-chasing system. It is striking that significant quantities of label appear in the larval gut contents as little as 0.5 hr after injection. This indicates that the labelled molecules are incorporated by the milk gland secretory cells, transported into the secretory vacuoles and moved the length of the tubules into the uterus and into the larval gut within 30 min of injection. Thus, during times of elaboration of secretion, the flux of amino acids is exceedingly high. The rapidity of movement of labelled molecules can be seen more clearly in Table 3. In the case of H\(^3\)-tyrosine, approximately 50% of the total label in the cells appears in the secretory vacuoles within 30 min after injection. An even higher percentage of the label appears in the secretory vacuoles (56–71%) within 30 min of injection of H\(^3\)-leucine. Thus, the \(t_{50}\), the time required for transport of 50% of the total cellular label to the secretory vacuole (see Kafatos and Kiortsis, 1971) must be approximately 30 min in the case of H\(^2\)-tyrosine and between 21 and 27 min in the case of H\(^3\)-leucine. For these calculations,
Table 2. Number of grains/100 µ² in autoradiographs of proximal milk gland (PMG), distal milk gland (DMG) and larval gut contents (LGC) at selected times during the first two pregnancy cycles of G. austeni. Figures represent the mean of ten counts on each of one or two animals, but where the density was greater than 150/100 µ², the number could not be determined. Range was less than ± 10%.

Grains/100 µ²

<table>
<thead>
<tr>
<th>Incubation time (hr)</th>
<th>H³-tyrosine</th>
<th>H³-leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0·5</td>
<td>2</td>
</tr>
<tr>
<td>6 days before 1st larviposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMG cytoplasm</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>PMG secretion</td>
<td>63</td>
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<tr>
<td>DMG cytoplasm</td>
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<td>38</td>
</tr>
<tr>
<td>DMG secretion</td>
<td>40</td>
<td>56</td>
</tr>
<tr>
<td>LGC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 days before 1st larviposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMG cytoplasm</td>
<td>29</td>
<td>60</td>
</tr>
<tr>
<td>PMG secretion</td>
<td>27</td>
<td>150</td>
</tr>
<tr>
<td>DMG cytoplasm</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td>DMG secretion</td>
<td>32</td>
<td>150</td>
</tr>
<tr>
<td>LGC</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4 days before 2nd larviposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMG cytoplasm</td>
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<td>56</td>
</tr>
<tr>
<td>PMG secretion</td>
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<td>150</td>
</tr>
<tr>
<td>DMG cytoplasm</td>
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<td>62</td>
</tr>
<tr>
<td>DMG secretion</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>LGC</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>2 days before 2nd larviposition</td>
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<td></td>
</tr>
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<td>PMG cytoplasm</td>
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<td>67</td>
</tr>
<tr>
<td>PMG secretion</td>
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<tr>
<td>DMG secretion</td>
<td>92</td>
<td>150</td>
</tr>
<tr>
<td>LGC</td>
<td>20</td>
<td>65</td>
</tr>
</tbody>
</table>

it must be assumed that the label is depleted in less than 21 min after injection and that the system is self-chasing. The evidence presented above suggests that these are reasonable assumptions.

Figs. 12, 13 and 14 are micrographs of autoradiographs, 0·5, 2 and 4 hr after injection with H³-tyrosine, showing the movement of label through the milk gland over the incubation period. Although each micrograph is of milk gland from a different fly, the diameter of the milk gland is identical in each case, indicating that the flies are of the same physiological age. The movement of label can be clearly seen in these figures, with label being approximately equally distributed between cytoplasm and secretory vacuoles at 0·5 hr but concentrated in the secretory vacuoles at 2 and 4 hr.

While the presentation of the data in this section has been directed primarily towards demonstrating the rapidity of the synthesis of the secretion, it is clear from Tables 1 and 2 that the rate of synthesis is not uniformly intense throughout the cycle of pregnancy. From these and similar data not presented here in detail, we have constructed a pattern of intense synthetic activity for the gland. This is summarized, along with information concerning the other tissues investigated, in the diagram in Fig. 15.
Table 3. The distribution of label in the cellular compartment of the proximal milk gland (PMG) and distal milk gland (DMG) expressed as a percentage of the total label present in the entire cell. These data are derived from autoradiographic grain counts of the glands from flies of the following ages: 6 and 4 days before the first larviposition and 4 and 2 days before the second larviposition. No significant difference was observed among flies of different ages:

<table>
<thead>
<tr>
<th>Incubation time (hr)</th>
<th>H(^3)-tyrosine</th>
<th>H(^3)-leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMG cytoplasm</td>
<td>DMG cytoplasm</td>
</tr>
<tr>
<td>0.5</td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>29, 18, 13</td>
<td>71, 82, 87</td>
</tr>
</tbody>
</table>

Fig. 15. Diagram summarizing the pattern of protein synthesis in female G. austeni during the first two pregnancy cycles in milk gland (MG), fat body (FB), oenocytes (OE) and epidermal cells (EP) as determined from autoradiography of flies injected with H\(^3\)-leucine or H\(^3\)-tyrosine. The horizontal lines indicate periods of intense synthesis.

Discussion

**Incorporation by fat body and oenocytes**

The period of maximum incorporation of H\(^3\)-leucine by both the fat body and oenocytes occurs just after the first larviposition (Figs. 1–3). This period coincides closely with the burst of RNA synthesis reported by Tobe and Davey (1974a) and suggests that the RNA synthesized at this time may act as the template for the large amounts of protein which are synthesized. The protein levels of the haemolymph begin to increase 3 days after the first larviposition (Tobe and Davey, 1974b) and it is possible that haemolymph proteins are being produced by the fat body and/or oenocytes at this time and these are subsequently released slowly into the haemolymph during the remainder of the pregnancy cycle.

It is also interesting to note that the levels of incorporation of H\(^3\)-leucine by the fat body and oenocytes at eclosion coincides precisely with the incorporation of H\(^3\)-uridine by the tissues (Tobe and Davey, 1974a). Although the fat body protein synthesized at this time may in part comprise the DNA synthetic machinery, at least some proportion of it probably represents haemolymph protein, since haemolymph protein levels begin to increase after day 2 post-emergence (Tobe and Davey, 1974b). Thus, a synthesis and slow release of haemolymph proteins may be occurring, similar to the situation suggested for the period just after the first larviposition. However, it should be noted that the oenocytes, which do not synthesize significant quantities of DNA, show high levels of both H\(^3\)-uridine and H\(^3\)-leucine incorporation and it is therefore possible that only a small proportion of the protein synthesized by the fat body at emergence is involved in subsequent DNA synthesis (see Tobe and Davey, 1974a).

The functional significance of the oenocytes remains obscure. Although these cells...
synthesize large quantities of RNA and protein, as indicated by the incorporation of H\textsuperscript{3}-uridine (Tobe and Davey, 1974a) and H\textsuperscript{3}-leucine, the fate of the protein is not known. Although it is tempting to suggest that the cells are producing haemolymph protein, there is no positive evidence to indicate that this is indeed the case.

Many roles have been suggested for the oenocytes in insects. Wigglesworth (1965, 1970) has implicated the oenocytes in the formation of cuticulin in *Rhodnius* while Locke (1969) has suggested a possible involvement of the oenocytes in steroid metabolism in *Calpodes*. However, the function of oenocytes of higher Diptera has not been extensively studied and although the work of Wolfe (1954) has conclusively demonstrated the involvement of oenocytes in lipoprotein formation for the cuticulin layer of the epicuticle of *Calliphora*, little other positive evidence exists on the other functions of oenocytes. Thomsen (1956) has suggested that oenocytes may be involved in some way with the female reproductive system since they are greatly enlarged in females. In the present study, there can be little doubt that a major product of the oenocytes is protein. Although there is an obvious correlation with the pregnancy cycles (Figs. 1–6), the exact role of this product in the metabolism of the adult female remains obscure.

The high levels of H\textsuperscript{3}-tyrosine incorporation by the oenocytes just after the first larviposition implicates these cells in the production of tyrosine-rich proteins. The increase in incorporation from 0·5 to 4 hr also suggests that these cells may be storing the newly synthesized protein for future release. It is possible that, prior to the elaboration of secretion by the milk gland, amino acids are stored in the form of proteins in the fat body and/or oenocytes. When the amino acids are required by the milk gland for the production of secretion, the proteins are broken down to the individual amino acids and released as either free amino acids or peptides. This may be particularly relevant for tyrosine due to its low solubility in aqueous solutions. Tobe et al. (1973) have shown that the oenocytes contain significant quantities of tyrosine-rich proteins and this is further evidence for the role of these cells in the storage of tyrosine-rich proteins.

The fact that the oenocytes do not appear to store tyrosine-containing proteins during the first pregnancy cycle can be readily explained by noting that high levels of tyrosine are incorporated and apparently stored during the first 4 days after emergence. Thus, the storage of these proteins during this period may reflect the requirement of the secretory milk gland cells for tyrosine during the first pregnancy cycle. It has been noted previously by Tobe and Davey (1974a) that the first pregnancy cycle seems to differ qualitatively from the subsequent cycles and it is possible that tyrosine is stored during the preovulation period in preparation for the first pregnancy cycle. However, during subsequent cycles, the oenocytes are capable of storing sufficient tyrosine at the beginning of each pregnancy cycle to meet the demands of the milk gland, at least in part. The amount of blood consumed during the preovulation period is considerably less than that consumed during the subsequent pregnancy cycles (Tobe and Davey, 1972) and it is possible that the fly requires the longer period to store sufficient tyrosine at this time than during the other pregnancy cycles.

The calculations presented earlier concerning the rates of utilization of amino acids by the milk gland of *G. austeni* during the pregnancy cycles indicate that there is an extremely rapid incorporation of injected radioactive amino acids used in the present study. In view of this rapid uptake of the labelled amino acids, several interesting points become apparent from the figures. In the case of H\textsuperscript{3}-tyrosine (Figs. 4–6), 2–3 days after the first larviposition the level of incorporation into the oenocytes increases between 0·5 and 4 hr and since it is known that the milk gland is actively elaborating secretion at this time, it may be assumed that the pool of H\textsuperscript{3}-tyrosine has been depleted within 0·5 hr after injection. Thus, the increased incorporation by the oenocytes cannot represent the simple uptake of the labelled tyrosine and implies that the tyrosine was taken up by some tissue, possibly the fat body, and subsequently released. Haemolymph protein, containing H\textsuperscript{3}-tyrosine, may represent the storage form at this time and the subsequent uptake of the amino acid, in either its free form or as a peptide or protein, may account for the increased incorporation. Once again, evidence suggests that the oeno-
cytes are intimately involved in tyrosine metabolism.

Depletion of the labelled tyrosine may not occur at all times during the pregnancy cycles. Thus, at times when the milk gland is either undeveloped (between days 0 and 4 after emergence: see Tobe et al., 1973) or not elaborating secretion (0–1 day after larviposition), the pool of labelled tyrosine may not be depleted for a longer period. The data presented in Figs. 4–6 suggest that depletion in newly emerged flies occurs between 2 and 4 hr after injection. On the other hand, the situation 1 day after larviposition may be similar to that 3 days after larviposition. That is, there may be storage of the labelled tyrosine, perhaps as haemolymph protein, and its subsequent utilization during the later stages of the incubation period.

It is significant that the levels of incorporation of both \( \text{H}^3 \)-leucine and \( \text{H}^3 \)-tyrosine are similar even though the specific activity of the injected tyrosine was 73 times that of the leucine. In view of the discussion above on the rates of utilization of the two amino acids, this is understandable since most or all of the labelled amino acids would be utilized within 0.5 hr after injection. The total amount of radioactivity injected into flies is identical for both amino acids, and this therefore provides further evidence that the labelled precursors are depleted within 0.5 hr.

The fact that levels of incorporation remain the same for most times observed over the three incubation periods, with the exception of those noted above, further emphasizes the fact that the fat body and oenocytes do not appear to engage in storage and subsequent release within a 4 hr period. Therefore, labelled proteins are either released immediately upon synthesis or are stored at low levels for release after the 4 hr period. This is indicated by the fact that the levels of incorporation do not drop between 0.5 and 4 hr.

Incorporation by epidermal cells

It is interesting that the levels of incorporation into the epidermal cells of both \( \text{H}^3 \)-leucine and \( \text{H}^3 \)-tyrosine rise dramatically around the time of the first larviposition. Whereas \( \text{H}^3 \)-leucine incorporation rises just before larviposition, \( \text{H}^3 \)-tyrosine incorporation does not rise until after larviposition. Label is observed in the endocuticle of flies 1 day after larviposition following injection with \( \text{H}^3 \)-tyrosine but a similar phenomenon is not observed in the case of \( \text{H}^3 \)-leucine. This suggests that tyrosine is being incorporated into cuticular protein while leucine is being incorporated into cellular protein and/or a cuticular lipoprotein which is removed during the embedding procedure.

The autoradiographic data presented in Figs. 9 and 10 also indicate that the protein synthesized by the epidermal cells is released at certain times provided that the assumptions made regarding the depletion of the labelled pools are correct. Thus, in the case of \( \text{H}^3 \)-leucine, there is an apparent decrease in incorporation over the 4 hr period, 5–6 days before the first larviposition and 1 day after the larviposition. The decrease in incorporation of \( \text{H}^3 \)-tyrosine is particularly pronounced just before and just after the time of the first ovulation. This coincides closely with the time of the observed histological deposition of endocuticle (Tobe and Davey, 1972) and indicates that the apparent decrease in incorporation in epidermal cells over the 4 hr incubation period in fact represents the release of cuticular proteins.

It is interesting that the apparent release of labelled proteins occurs at different times in the case of \( \text{H}^3 \)-leucine and \( \text{H}^3 \)-tyrosine. Thus, release of tyrosine-labelled protein coincides with the time of maximum endocuticle deposition while the release of leucine-labelled protein occurs, at least in the first pregnancy cycle, at the time when the endocuticle is beginning to stretch (Tobe and Davey, 1972).

Incorporation by the milk gland

Tobe and Davey (1972) have suggested that the preovulation period represents a period of post-emergence growth and differentiation for the female fly. In addition, the preovulation period must represent a time of storage for the fly, in preparation for the first pregnancy cycle. Thus, although flight muscle development (Bursell, 1961), oocyte maturation and cuticle deposition (Tobe and Davey, 1972) occur during this period, the haemolymph protein level almost doubles during this period (Tobe and Davey, 1974b) and the fat body also increases in size (Tobe et al., 1973). During the pregnancy
cycles, the fly is also depositing cuticle and maturing oocytes so that the major differences between the preovulation period and subsequent pregnancy cycles is the development of flight musculature in the preovulation period and the elaboration of milk secretion during the pregnancy cycles. It therefore appears that the production of milk secretion represents the major metabolic drain on the nutrients of the female during adult life.

The formation of zymogen by the galea of the silk moth has been extensively investigated by Kafatos and co-workers (see Kafatos (1969) and Kafatos and Kiortsis (1971)) and represents, in some respects, an analogous system to the milk gland of *G. austeni*, in that large quantities of protein product are produced by specialized cells of ectodermal origin over short periods of time. However, the two systems differ in that the galeal cells are active for only a brief period, just before adult eclosion, while milk gland cells undergo cyclic secretion throughout adult life. Also, the galeal cells produce only one major protein for export while it is not known how many proteins the milk gland cells produce for export. However, comparison of the two systems reveals some interesting points. Cytoplasmic product in galeal cells is transported into the secretory vacuoles for a period of about 6 days and according to the results of Kafatos and Kiortsis (1971), the $t_{50}$ for the galeal cells varies from 38 to 74 min depending upon their age. Thus the $t_{50}$ suggested for the secretory cells of the milk gland of *G. austeni* (21–30 min) is considerably shorter than that of the galeal cells, in fact approaching half the value. It should be noted that the estimated $t_{50}$ of milk gland cells is probably over-estimated in view of the fact that some finite time is required for movement of the label in the haemolymph at the site of injection (the thorax) into the abdomen. The experiments of Kafatos and Kiortsis (1971) were conducted in vitro and thus under their conditions, the time required for labelled precursors to reach the target organ was minimal. The effective pulse duration in *G. austeni* has been previously calculated and in the case of H$^3$-tyrosine, is only limited by the movement of labelled molecules into the abdomen since the rate of uptake of tyrosine is sufficient to deplete the labelled pool in a matter of minutes. Of course, the size of the free tyrosine pool influences the rate of uptake of labelled molecules but in view of the extremely rapid uptake of tyrosine and the low solubility of free tyrosine in the haemolymph (if the haemolymph volume is 5 µl, there can be no more than 2 µg of free tyrosine in the haemolymph), all the labelled molecules are probably utilized well before 30 min after injection.

The duration of pulse for H$^3$-leucine is probably somewhat longer than that for H$^3$-tyrosine, due to the larger amount of H$^3$-leucine injected, but the results suggest that the label is depleted within 30 min of injection and thus the pulse time must also be less than 30 min. The slightly longer duration of pulse in the present experiments, when compared with that of Kafatos and Kiortsis (1971) is probably offset by the delay in availability of labelled molecules to the abdominal tissues of *G. austeni* following injection and by the smaller quantities of labelled molecules used in the present study. It is therefore suggested that the $t_{50}$ of the secretory milk gland cells is considerably less than that of galeal cells. Jamieson and Palade (1967a, b) have extensively studied the kinetics and route of secretion in pancreatic acinar cells and have shown that the $t_{50}$ for the pancreas acinar cells is 63 min. If production of secretion follows a similar course in the milk gland of *G. austeni* it is apparent that the $t_{50}$ is considerably less than that of the highly efficient pancreatic system.

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References


